REMARKS/ARGUMENTS

The Final Office Action dated 6/19/2007 rejected claims 1-8 and 18; claims 9-17, 20 and 21 were withdrawn from consideration. No claims were allowed. By Amendment dated 8/15/2007, claims 1, 4, and 18 were amended and claim 5 was canceled. This amendment was not entered. However, Applicants filed an RCE dated 9/19/2007. In view of the RCE, this supplemental amendment treats the 8/15/2007 amendment as having been entered. By the present Supplemental Amendment, the specification and claims 1 and 7 are amended, and claims 9-18 and 20 -21 are canceled, claim 19 having been previously canceled.
In view of the above amendments, the accompanying Exhibits and the following remarks, the Examiner is respectfully requested to withdraw the rejections and allow claims 1 -4 and 6 – 8, the only claims remaining in this application.

The courteous interview granted applicants' attorney, Bertrand Rowland, is gratefully acknowledged. The Interview Summary dated 10/17/2007 from the Examiner is acknowledged under 37 CFR 1.133 as recording the substance of the interview, except that the interview was not with David Aston.

By the present Supplemental Amendment, the claims have been amended to more closely relate to the working exemplification. Support for the linker language, -R-Z, etc., is found in the formula at page 6, and in the following paragraphs [00025], relating to R, and [00026], relating to Z. Antecedent support for the 200 times affinity of the binding protein has been introduced into the specification, having been present in the original claims, e.g. claims 1 and 9. The 200-fold greater affinity is an arbitrary value based on the report by Iwasaki, of record, that the protein had a 1000-fold higher affinity than the natural receptor. This value provides a lower limit in the event that the binding protein becomes partially denatured or the expression of the protein in a host diminishes the affinity. The 200-fold increase in affinity over the natural receptor is sufficient for an assay. Furthermore, claim 1 is limited to the "sponge" binding protein, so the minimum of 200-fold affinity is a safety factor.

Accompanying this response are exhibits describing the commercialized version of the subject assay. Exhibit 1 is a book chapter by the inventors and their coworkers, "Homogeneous

Fluorescent Polarization Assay for Inositol 1,4,5-Trisphosphate (Ins P_3)," Chapter 20, ed. Lisa Manor, Handbook of Assay Development in Drug Discovery, CRCnetBase. Exhibit 2 is a Power Point presentation comparing evaluating the subject assay as to binding to other phosphate inositols. In the development of the assay, it was found that the IP_3R, which is about a 300kD protein, breaks down with time and could not be isolated as a pure protein. This is consistent with the failure of Hirata to use a pure protein for the study, rather than a complex composition of microsomes.

Applicants adopt and repeat in this response the arguments made in the response to the FINAL REJECTION of June 19, 2007. In the Advisory Action the Examiner indicated that the amendments made in that response raised new issues and would not be entered. With the filing of the Request for Continued Examination, those amendments are now treated as entered, and new amendments are submitted.

At the interview, it was pointed out that the present claims are more restricted in scope than the prior claims in limiting the linker to the fluorescent label. It was the Examiner's position that Hirata had prepared IP_3 analogs bound at the 2-position. Hirata was concerned with whether his analogs would react with IP_3-5-phosphatase. It is reported that the D-isomer is a potent inhibitor. Substantial differences in binding were observed between the phenyl and cyclohexyl derivatives in their binding to the enzyme.

In testing the analogs with IP_3-3-kinase, in contrast to the experience with the phosphatase, "the kinase activity was highly selective to D-isomers for both analogs." Therefore, the binding pattern of these enzymes was substantially different.

Hirata also tested rat cerebellum microsomal fractions. In this assay a radioactive IP_3 was used to compete with the analogs. The D-isomers inhibited the binding of the radioactive IP_3, while the L-isomers were relatively inactive. Figure 6 shows that the analogs are not very effective in inhibiting radioactive IP_3 from binding to the rat cerebellum microsomal fraction. About 1nM of the radioactive IP_3 is employed, while 50% inhibition requires 10nM of the D-isomers, including cold IP_3. Clearly, there is an inconsistency in this data. One might think that the microsomal fractions had higher capacity than the 1nM concentration of the hot IP_3.

However, the fact that there is some inhibition at a 1:1 concentration suggests that the microsomal capacity is not much greater than for the 1nM of hot IP_3. It is then difficult to resolve why it should take a 10-fold greater concentration of cold IP_3 to reduce the binding of the hot IP_3 to 50%. A rational explanation would be non-specific binding in view of the complexity of the composition used for binding. The conclusion should be that one cannot assume that the inhibition observed is of the IP_3 receptor, but of non-specific binding. Furthermore, it is noted that a log scale is used which tends to smooth out the curve. The graph would look very different if the log scale had not been used. Remarkably, with the cold IP_3 at 100 times the hot IP_3 concentration, one still is getting only 25% inhibition.

Hirata states that after incubating with the IP3 and filtering the microsomal fraction the filter is washed twice with 2ml of 50mM Tris-HCl. How vigorous was the washing, what was removed from the filter, how efficiently non-specific binding IP_3 was removed is totally left to conjecture. Considering the ionic nature of IP_3 and the presence of the amino group, it is very likely that there was extensive non-specific binding. The results support this conclusion.

What may one conclude from Hirata? There is clearly some effect of the 2-substituted analogs, but it is not clear for what they are competing with the hot IP_3. What one must conclude is that there is a substantial amount of non-specific binding to which IP_3 binds and the cold IP_3 and analogs can compete for these sites. It is submitted that Hirata does not teach that the 2-derivatives can successfully compete for the IP_3 receptor.

There is the further consideration that the "sponge" protein is not the IP_3 receptor. Rather it is a fragment of the receptor that has 1000 fold greater affinity for IP_3 than the intact receptor. Furthermore, the sponge protein lacks the N-terminal section of the IP_3R and replaces these amino acids with GST, as the expression product without the GST is "not efficiently expressed as a soluble active form." Clearly, one is concerned with conformational properties that affect the folding of the protein as well as its ability to bind ligands. Iwasaki does not determine the specificity of this sponge protein. While it has a 1000-fold higher affinity for IP_3 than the natural receptor, it is not tested for its specificity. It is not known to what degree it binds other phosphate derivatives of inositol. In the absence of any evidence of specificity, there was not

reasonable certainty that the sponge protein would be satisfactory. Iwasaki uses his protein to compete for IP_3 with the IP_3R, but does not investigate whether other phosphate derivatives of inositol are also bound.

In reviewing Hirata, there is no support for direct competition between Hirata's analogs and IP_3 for IP_3R. The data just do not support such conclusion. Rather, in view of the ionic character of IP_3 and its analogs and the uncertainty respecting the microsomal fraction as to what IP_3 binds, there is no evidence that one could develop an assay for IP_3 that would have a satisfactory dynamic range, be highly sensitive and specific with sensitivity increasing with diminishing concentration of IP_3. See, paragraph [00055].

While at first glance it would seem that Hirata and Iwasaki point to reagents for developing an IP_3 assay, the fact is that on closer scrutiny they do not provide the information necessary to have a reasonable expectation of success. Hirata provides evidence which cannot be reconciled with a simple competition between his analogs and IP_3 for IP_3R. Iwasaki, while indicating that he has a binding protein with very high affinity for IP_3, tells us nothing about the specificity of the protein. The difficulty in expressing the protein, the need to have a different N-terminal region from the natural region, and the high affinity as compared to the natural IP_3R, leave one concerned as to the utility of the protein in an assay.

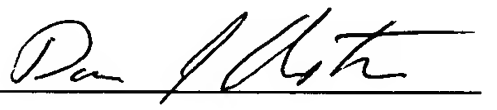
Attorney Docket No. 3817.14-1
Customer No. 23308

Conclusion

Applicants request that this amendment to the claims and specification be entered as supplemental to the previous amendment dated 8/15/2007, now presumably entered by virtue of the RCE filed 9/17/2007, and requests reconsideration and allowance of claims 1-4, and 7-8 for the reasons advanced above. If the Examiner believes that the prosecution of the application could be expedited by a telephonic interview, the Examiner is requested to call Bertram Rowland, Reg. no. 20,015, at 650 344 4674.

Respectfully submitted,

PETERS VERNY, LLP

By 

David J. Aston, Reg. No. 28,051
Tel.: (650) 324-1677
Fax: (650) 324-1678